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# Effects of essential oil supplementation of a corn silage based diet fed to lactating Holstein dairy cows

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# EFFECTS OF ESSENTIAL OIL SUPPLEMENTATION OF A CORN SILAGE BASED DIET FED TO LACTATING HOLSTEIN DAIRY COWS

BY

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THESIS

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## DEDICATION

To my parents, for always believing in me, and their never-ending support, encouragement, dedication, and unconditional love throughout my entire life. I am forever thankful.

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It is so hard to put into words all the thanks I want to say to my parents. They have been the model parents throughout my entire life. They never once gave up on me. They always supported my decisions, even when I knew they disagreed. They have allowed me to determine my own path in life, and I am very thankful to them for instilling morals and values in me early on in life so I had the opportunity to become the person I am today. It is entirely because of them that I have made it this far. Their financial support, encouragement, patience and dedication have helped me obtain my dreams and educational goals. The unconditional love they have shown one another and me has not gone unnoticed. I only hope that one day I can reciprocate all they have done for me. I thank them both so much and from the bottom of my heart.

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**ABSTRACT**

ESSENTIAL OIL SUPPLEMENTATION OF A CORN SILAGE BASED DIET DEFICIENT IN RUMEN UNDEGRADABLE PROTEIN FED TO LACTATING HOLSTEIN DAIRY COWS

by

Carly Crawford

University of New Hampshire, September, 2006

Thirty multiparous early lactation Holstein cows were used in a randomized complete block design to determine whether the addition of VERTAN (a specific blend of essential oils, major components: thymol, eugenol, vanillin and limonene) to corn silage based diet would increase alter protein metabolism in the rumen and increase milk yield and alter milk composition. Dietary treatments were 0 (control) and 0.08% VERTAN in diet DM. The pretreatment diet (fed 0 to 20 DIM) contained (DM basis) 32.1% corn silage, 14% grass silage, 7.7% alfalfa hay, 0.53% grass hay, 19.4% finely-ground corn, 1.8% beet pulp, 1.8% citrus pulp, 3.6% soybean hulls, 2.1% ProvAAl Elite™, 0.38% molasses, 10.5% soybean meal, 0.34% urea, 1.9% Megalac™, and 3.54% vitamin premix. The experimental control diet (fed 21 to 105 DIM) contained (DM basis) 29.8% corn silage, 14.9% grass silage, 7.2% alfalfa hay, 0.11% grass hay, 21.5% finely-ground corn, 1.6% beet pulp, 1.6% citrus pulp, 4.3% soy hulls, 0.79% molasses, 11.9% soybean meal, 0.43% urea, 0.06% Smartamine M™, 2.5% Megalac™, and 3.4% vitamin premix. The diets were formulated to meet NRC (2001) requirements for energy

and all nutrients except RUP. Cows were fed and milked 3 times daily. Milk samples were taken at every milking on two different days in wk 3 of lactation (covariate period) and on 1 day every week during wk 4-15 of lactation (treatment period). Blood samples were taken twice weekly during wk 3-15. Intake of DM, milk yield, milk composition, blood urea N, and body weight and body condition scores were not affected by essential oil supplementation. Two multiparous lactating Holstein cows fitted with a ruminal cannulae were used in a switchback design (each cow received each dietary treatment twice) for collection of rumen fluid. Experimental periods were 4-wk in length. Rumen samples (n=16) were collected during wk 4 in each period. Sampling occurred over 2 consecutive days and was such that a sample was collected every 2-h in a 24-h day. Ruminal pH showed no significant differences between treatments. Essential oil supplementation on rumen ammonia N concentrations over a 24-h period had a significant hour by treatment affect. The results of this study suggest that more research needs to be done on dose levels of essential oils and diet effects on essential oils.

**keywords:** lactating cows, essential oils, corn silage

# CHAPTER I

# REVIEW OF THE LITERATURE

**Abbreviation Key:** **AA** = amino acids, **CP** = crude protein, **EO** = essential oils, **FA** = fatty acids, **HAP** = hyper-ammonia producing, **RDP** = rumen degradable protein, **RUP** = rumen undegradable protein, **VFA** = volatile fatty acids.

## Introduction

Plants have fascinated man for centuries because of their many beneficial uses. Plants have been used for medicinal purposes dating as far back as Hippocrates in the late fifth century B.C. He mentioned 300 to 400 medicinal plants used by man (Schultes, 1978). Borris (1996) estimated that there are 250,000 to 500,000 species of plants on Earth and more have probably been discovered since then. Today plants are used by man for their medicinal, preservative and antiseptic properties. Prior to the discovery of antibiotics, plant extracts, such as essential oils (EO), were used widely for human pharmaceutical purposes. Once the power of antibiotics was discovered, research investigating the action of EO declined. However, with increased awareness of antibiotic resistance, interest in plant extracts for medicinal purposes has recently increased. The public has become more aware of problems of over-prescribing and misuse of traditional antibiotics in themselves as well as in animals. Due to the recent ban of growth-promoting antibiotic use in animal feeds in Europe (Wallace, 2004), and the recent rise in

the number of organic animal farms in the U.S., finding alternatives to antibiotics for use in animal production is critical.

## The Evolution of Essential Oils

Resistance to antibiotics is the driving force behind the ongoing research to find natural alternatives to antibiotics (Wallace, 2004). The products that have been investigated, and are considered 'natural', are probiotics, prebiotics, enzymes, organic acids and secondary plant compounds, including EO (Wallace, 2004). Secondary plant compounds serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores (Cowan, 1999).

There are several classes of secondary plant compounds including phenolics, polyphenols, flavonols, tannins, saponins and EO. Essential oils provide the plant with its odor and flavor (Cowan, 1999) and provide man with antiseptics and preservatives (Wallace, 2004). Essential oils have been shown to be active against certain bacteria (Busquet et al., 2005a; Busquet et al., 2005b; Elgayyar et al., 2001; Imai et al., 2001; Shapiro et al., 1994) and saponins, which are present in *Yucca schidigera*, a common carrier for EO products, have been shown to be active against protozoa (Hristov et al., 1999; Wang et al., 1998). A detailed discussion on the action of EO on certain bacteria will follow.

## Extraction of Essential Oils

Essential oils are present in most parts of the plant, including the leaves, roots, bark and flower and they accumulate in specialized structures such as oil cells, grandular trichomes, and oil or resin ducts (Simon, 1990). For example, the roots of the ginseng plant contain the active saponins and EO, and eucalyptus leaves are harvested for their

EO and tannins (Cowan, 1999). Chemically, EO are called terpenes because their general structure is $C_{10}H_{16}$ and they occur as diterpenes ($C_{20}$), triterpenes ($C_{30}$) and tetraterpenes ($C_{40}$), as well as hemiterpenes ($C_5$) and sesquiterpenes ($C_{15}$) (Cowan, 1999; Simon, 1990). When additional elements are present in the compound, usually oxygen, they are termed terpenoids (Cowan, 1999). Terpenoids are synthesized from acetate, which also synthesize fatty acids (FA) (Cowan, 1999). Terpenoids differ from FA in that terpenoids contain extensive branching and are cyclized (Cowan, 1999). It is a common misconception that EO are FA because of the word 'oil', but they are not similar structurally or functionally to oils (Cowan, 1999).

There are a number of methods to extract the various secondary compounds out of plant parts. At home, plants can be dried and ingested as teas (plants steeped in hot water), tinctures (plants in alcoholic solutions), or inhaled by steam from boiling suspensions of the plant parts. Dried plant parts can also be mixed with petroleum jelly and applied externally (Brantner and Grein, 1994; Thomson, W.A.R., 1978).

For research or commercial purposes, extraction is done differently. Since EO are, for the most part, volatile, they can be extracted via water and steam distillation, direct steam distillation, and solvent extraction (ATSA, 1968; Guenther, 1972; Heath, 1981; Losa, 2001; Sievers, 1928). However, extraction must be monitored carefully because EO are complex mixtures of organic constituents and some may undergo chemical alterations when subjected to high temperatures (Simon, 1990). In these cases, organic solvent extraction is required to be sure no decomposition or changes have occurred. Decomposition or alteration could alter the aroma, fragrance and function of the end-product (Simon, 1990). A more costly extraction method is the use of

supercritical $CO_2$ which yields very high quality oils for commercial use (Simon, 1990). Non-volatile EO are extracted also via solvent extraction, although the process is more difficult and complex than the extraction of the volatile EO (Simon, 1990).

In the U.S., the majority of EO are by-products of industrial processes. For example, citrus EO are extracted from the peel which contains the oil sacs, or glands, located in the outer mesocarp of the fruit (Matthews and Braddock, 1987). These glands need to be expressed by pressure or mechanical rasping in order to harvest the EO (Matthews and Braddock, 1987). The wood and pulp manufacturing industries also produce a large quantity of EO (Lawrence, 1979). Mint and dill EO are the most abundant in the U.S. and are obtained via steam distillation. Most other plants that contain EO are grown and imported from the temperate zone (Simon, 1990).

**Uses for Essential Oils**

Essential oil use for medicinal or pharmaceutical purposes has recently regained attention from the scientific community because of the increasing problem of antibiotic resistance (Wallace, 2004). Bacteria, such as *Escherichia coli* O157, have become resistant to certain antibiotics and there have been recent appearances of new, highly resistant verotoxigenic strains of *E. coli* (Wallace et al., 2002). Imai et al. (2001) found that EO are bactericidal to certain bacteria that are resistant to antibiotics.

Certain disease causing bacteria can be transmitted from animals to humans (Wallace et al., 2002). One example would be bacterial contamination of meat through mishandling at slaughter. Elder et al. (2000) found that *E. coli* O157 is present in the feces of 28% of cattle, and 11% of cattle presented for slaughter carry *E. coli* O157 on their hides. Varel and Miller (2001) concluded that there is a correlation between fecal

prevalence and carcass contamination. Essential oils may be useful in preventing proliferation of harmful bacteria on carcasses at slaughter. This application will be discussed in depth later in the paper.

Another sign of possible antibiotic resistance is the fact that *Staphylococcus aureus*, which causes frequent hospital infections among other illnesses, is resistant to methicillin (Imai et al., 2001). This is by far, not the only bacteria resistant to an antibiotic. Others include *E. coli* O157:H7, *Lactobacillus plantarum*, *Listeria monocytogenes*, *Salmonella* Typhimucrium, and *Yersinia enterocolitica* (Elgayyar et al., 2001). Peppermint and spearmint oils at 800 μg/ml were shown to be bactericidal to bacteria that were resistant to antibiotics (Imai et al., 2001).

There are many uses for EO, and the research conducted to investigate their uses has lead to their potential use in livestock feeding. In 2004, the FDA approved the use of most EO in livestock and categorized them as GRAS (generally recognized as safe). These uses include food preservation, odor control, anti-bacterial, anti-protozoal, anti-fungal, and anti-allergen (Busquet et al., 2005a; Busquet et al., 2006; Elgayyar et al., 2001; Imai et al., 2001; Varel and Miller, 2001; Wang et al., 1998). Due to the fact that secondary plant compounds are anti-bacterial and anti-protozoal, it has been speculated that they can be used to manipulate ruminal fermentation to make the cow more efficient in converting feed to product (Wallace et al., 2002).

There are more than 1,340 plants known to be potential sources of antimicrobial compounds, but only a select few have been studied (Wilkins and Board, 1989). The EO that have been widely investigated are: anise, basil, carvacrol, chamomile, cinnamon, eucalyptus, garlic, oregano, peppermint, sage, spearmint, thyme, and *Yucca schidigera.*

Iami et al. (2001) investigated the effects of the EO of peppermint, spearmint, and Japanese mint on the pathogenic bacteria *Helicobacter pylori*, *Salmonella enteritidis*, methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *S. aureus*, and *Escherichia coli* O157:H7. The major constituents of peppermint are menthol, menthone, cineole, and neomenthol and the major constituents of spearmint are carvone, l-menthol, and myrcene (Fujita and Nezu, 1980). In previous experiments the authors concluded that peppermint inhibited bacteria (Shapiro et al., 1994) and fungi (Dikshit and Husain, 1984) and had anti-allergen activity (Arakawa et al., 1992). Imai et al. (2001) confirmed the antibacterial activities of mint EO and their constituents against many strains of pathogenic bacteria, including antibiotic-resistant bacteria.

Elgayyar et al. (2001) investigated the use of several EO in food preservation. Their effects on the growth of four Gram-negative bacteria, three Gram-positive bacteria, and three fungi were tested. Inhibition was measured using a scale that measured the zone of inhibition around the antibiotic disks ($\geq$ 28mm zone of inhibition is strongly inhibitory; < 28 to 16 mm zone of inhibition is moderately inhibitory and so on). While the bacteria were resistant to antibiotic discs placed in the media, oregano, containing primarily thymol and carvacrol, completely inhibited the growth of three of the four Gram-negative bacteria, two of the three Gram-positive bacteria, and all three of the fungi tested. Most of the other oils tested showed inhibition of one or more of the bacteria. Carrot oil was the only oil tested that showed no inhibition toward any of the bacteria. Although this study focused on the use of EO for food preservation, the authors noted that more research is necessary to evaluate flavor, chemical changes and antimicrobial effects of EO in the food system. A similar study was conducted by

Marino et al. (2001) to determine the antimicrobial activity of EO on nine strains of Gram-negative bacteria and six strains of Gram-positive bacteria. It was concluded that the bactericidal effect of oregano EO, and possibly other EO, was irreversible: i.e., recovery after enrichment was limited.

Oral hygiene is another area where EO are used to inhibit the growth of bacteria. Essential oils have been used for mouth odor and pain for thousands of years (Shapiro et al., 1994). Shapiro et al. (1994) conducted a study to prove the importance of EO use in oral hygiene. The study tested several plant extracts and EO components and concluded that Australian tea tree oil, peppermint oil, sage oil, and thymol are all inhibitory toward oral bacteria.

**Use of Essential Oils in Ruminant Diets**

Experiments conducted testing the effects of one EO against one bacterial species (Elgayyar et al., 2001; Imai et al., 2001; Shapiro et al., 1994) prompted researchers to evaluate the effects of a mixture of EO on a mixture of bacterial species, specifically, ruminal bacteria. Oh et al. (1968) examined the effects of EO on ruminal bacteria and determined that there was general inhibitory activity across a range of plant materials, with vinegar weed being the most potent. Previous work by the same authors indicated that individual oils have different effects on mixed ruminal bacteria (Oh et al., 1967). Some of the EO were less toxic and stimulatory to microbial activity, while others were more toxic and inhibitory. Nagy and Tengerdy (1968) investigated whether ruminal bacteria could adapt or become resistant to EO. The authors found that the sensitivity of ruminal bacteria to EO of Big Sagebrush was the same in captive deer as it was in wild deer, suggesting that ruminal bacteria did no adapt to EO. Recently, studies have shown

conflicting results in terms of the ability of rumen microbes to adapt to EO in the rumen (Cardozo et al., 2004; McIntosh et al., 2003; Molero et al., 2004).

Due to concern of antibiotic residues in milk and the recent ban on ionophore feeding in Europe, interest has increased to find natural products that beneficially manipulate ruminal fermentation. Newbold et al. (2004) investigated the effects of CRINA (Ruminant; Akzo Nobel Surface Chemistry Ltd., Hertfordshire, United Kingdom) on ruminal protein metabolism in 4 ruminally cannulated sheep fed a 60:40 grass silage:concentrate diet. CRINA is a specific blend of EO including thymol, guajacol and limonene and was added to the diet such that the sheep were fed 110 mg/d. Rumen fluid was taken 2 h after the morning feeding and was used to determine proteolytic, peptidolytic and deaminative activities of rumen microorganisms. Casein acid hydrolysate was used as the substrate. Proteinase activities were measured as the release of label from [$^{14}$C] labeled casein, as demonstrated by Wallace (1983). Essential oils had no effect ($P > 0.05$) on ruminal pH or ammonia concentrations. The degradation of $^{14}$C labeled casein, dialanine or penta-alanine was not affected by CRINA, but deaminase activity was inhibited by 24% ($P < 0.05$). Microbial protein production was not affected.

Russell et al. (1991) determined that the deamination of amino acids (AA) is carried out by two distinct bacterial groups: a large number of bacteria with a low specific activity of ammonia production and a second population of bacteria present in low numbers but each possessing a very high specific activity of ammonia formation. The latter are called hyper-ammonia producing (HAP) bacteria. Monensin is known to inhibit all of the HAP species, while EO only inhibit two – *C. sticklandii* and *P.*

*anaerobius*. This may be why monensin was more effective at inhibiting ammonia production in the rumen than EO (McIntosh et al., 2003).

## The Mode of Action of Essential Oils

Secondary plant metabolites contain the antimicrobial activity of plant extracts. These include saponins (present in extracts of Yucca schidigera or Trigonella foenum graecum), terpenoids (such as carvacrol, carvone, or thymol), and phenylpropanoids (like cinnamaldehyde, eugenol and anethol). These secondary plant metabolites are present in the EO fraction of many plants (Busquet et al., 2006).

The mode of action of secondary plant metabolites has been discussed by several scientists (Busquet et al., 2006; Cardozo et al., 2005; Marino et al., 2001; McIntosh et al., 2003). Essential oils rich in phenolic compounds (e.g. thymol and carvacrol) are more effective as antimicrobials in comparison to their non-phenolic secondary plant metabolite counterparts (Helander et al, 1998; Ultee et al., 2002). In general, Gram-negative bacteria seem to be less sensitive to EO compounds than Gram-positive bacteria (Davidson and Naidu, 2000). This effect is most likely due to the presence of an outer membrane on Gram-negative bacteria, which gives a hydrophilic bacterial surface that acts as a strong impermeable barrier, thus providing some degree of protection from certain EO (Nikaido, 1994). Helander et al. (1998) reported that thymol and carvacrol can inhibit Gram-negative bacteria by disrupting the outer membrane. This inhibitory activity was thought to be due to the presence of the phenolic group. Ultee et al. (2002) suggested that carvacrol kills Gram-negative cells by disrupting their cell membranes, this results in a decreased proton-motive force across the cell membrane and a reduction in the synthesis of ATP, slowing microbial growth and eventually causing cell death.

Carvacrol is a non-specific antibacterial compound. It inhibits both Gram-positive (mostly acetate- and butyrate-producing bacteria) and Gram-negative bacteria (mostly propionate-producing bacteria). Because carvacrol is non-specific, it may be undesirable in the rumen (Busquet et al., 2006).

Skandamis and Nychas (2000) observed bacteria that seem to be more susceptible to the effects of EO at low pH. This may be related to the dissociated (hydrophilic) or undissociated (hydrophobic) status of the active molecules. Interaction of the bilayer cell membrane can only occur with the undissociated, hydrophobic form of the molecule. Acids tend to become undissociated and more hydrophobic at low pH, therefore interacting more easily with cell membranes where they exert their antimicrobial effect (Cardozo et al., 2005). The antimicrobial effect of the EO of thyme, cinnamon, and clove bud increased as pH decreased from 6.5 to 5.5 (Juven et al., 1994). Therefore, it was concluded that there is a relationship between ruminal pH and the antimicrobial effect of plant extracts (Cardozo et al., 2005).

## Effects of Essential Oils on Mixed Ruminal Microorganisms

Overfeeding protein is undesirable because protein is often the most costly nutrient in a ration, and there will be inefficient conversion of feed protein into milk protein by the animal. The NRC (2001) separates dietary crude protein (CP) into two distinct fractions: rumen degradable protein (RDP) and rumen undegradable protein (RUP). Rumen degradable protein is the fraction of dietary CP that is potentially degradable in the rumen. It consists of dietary true protein and non-protein N compounds such as free AA and supplemental urea. Rumen degradable protein is used by rumen microorganisms for the synthesis of microbial protein. The RDP that is true protein is

degraded in the rumen by microorganisms into oligopeptides, then to dipeptides, then to AA, which can then be converted to microbial protein, or deaminated to yield ammonia and C skeletons. The ammonia and C skeletons are substrates for AA synthesis by rumen bacteria. Mixed rumen bacteria use significant amounts of ammonia for AA and protein synthesis. Ammonia produced in excess of bacterial requirements will be absorbed through the rumen wall and converted to urea in the liver. Upon its release from the liver to the blood, it is either recycled back to the rumen (via saliva or passive transfer across the rumen wall) where it again is hydrolyzed to ammonia, or extracted by the kidney and excreted in the urine (NRC, 2001). A goal in ruminant nutrition is to provide an amount of RDP that meets, but does not exceed, the N requirements of rumen microorganisms for optimal efficiency of microbial protein synthesis, and to obtain the desired animal productivity with the smallest amount of dietary CP (NRC, 2001).

Rumen undegradable protein is that fraction of dietary true protein that escapes, or resists ruminal degradation, and passes through the rumen to the small intestine intact. The benefits of meeting but not exceeding protein requirements are decreased feed costs, more space in the diet for other nutrients such as fermentable carbohydrates, increased conception rates, and decreased N waste (NRC, 2001). With increased concern of environmental N contamination by livestock production units, alternatives have been investigated to decrease ammonia production in the rumen, thus decreasing N excretion. One such alternative is feeding EO.

**Comparison to Ionophores**

As stated previously, HAP bacteria in the rumen specifically generate ammonia from AA (Russell et al., 1991). These bacteria represent only 1% of the rumen microbial population, yet they possess a very high specific activity of ammonia formation from AA (Wallace et al., 2002). In a study using sheep, Wallace et al. (2002) determined that the number of total ruminal bacteria was unaffected by EO, but the number of HAP bacteria decreased by 77% in sheep fed a low-protein diet. Essential oils are not as effective at preventing deamination of AA by rumen bacteria as monensin is, but nevertheless, even a small decrease in the rate of ammonia production may be beneficial, so the suppression of the HAP bacteria would be expected to be significant (Wallace et al., 2002). Others have also demonstrated that EO decrease deamination of AA (Castillejos et al., 2006; McIntosh et al., 2003; Newbold et al., 2004).

Ionophores are used in ruminant nutrition to prevent or decrease the incidence of digestive upsets (Bergen and Bates, 1984; Chalupa et al., 1980) and to reduce energy (in the form of methane) and N (in the form of ammonia) losses (McGuffey et al., 2001). It seems as though EO act in a similar way to ionophores, although to a much lesser extent, within the rumen (Busquet et al., 2005a; Busquet et al., 2005b; Busquet et al., 2006; Cardozo et al., 2004; Cardozo et al., 2005; McIntosh et al., 2003; Newbold et al., 2004; Wallace et al., 2004; Wang et al., 1998). To benefit rumen conditions, volatile fatty acid (VFA) concentrations should increase or stay the same and the acetate to propionate ratio should decrease when ionophores or EO are supplemented (Cardozo et al., 2005). Lila et al. (2003) concluded that adding 3.2 g/L of sarasaponin (a group of steroidal glycosides extracted from the *Yucca schidigera* plant) in vitro decreased acetate concentration and

decreased the acetate to propionate ratio. Similar results were found by Castillejos et al. (2006) using thymol, eugenol, limonene, guajacol, and vanillin. Incubations were conducted using rumen fluid from 2 lactating dairy cows fed a 60:40 alfalfa hay:concentrate diet. Limonene, guajacol, and vanillin decreased acetate concentrations compared to no EO supplementation and limonene inhibited deamination of AA at 500 mg/L.

Similar results have been observed in vivo. Hristov et al. (1999) fed 6 ruminally cannulated Angus heifers an alfalfa silage:barley grain (39:61) diet. The heifers were supplemented with 0, 20, or 60 g/d of *Yucca schidigera. Yucca schidgera* supplementation decreased the acetate to propionate ratio and decreased ammonia concentrations 2-h after feeding and again at 4-h after feeding. Protozoa numbers were reduced by 42% in ruminal fluid from heifers receiving 20 g/d of *Yucca schidigera.*

Benchaar et al. (2004) fed 0, 2 or 4 g/d of VERTAN (a specific blend of EO), or 220 mg/d of monensin to 20 steers and 20 heifers (Angus x Hereford). The animals were fed a TMR comprising 75% grass/legume silage and 25% rolled barely. Intake of DM was not affected by EO supplementation but it was 10% lower ($P < 0.01$) in cattle supplemented with monensin. Daily weight gains did not differ between supplemented and control animals. Feed efficiency was decreased by the addition of 2 g/d EO, but increased when the diet was supplemented with 4 g/d EO.

**Essential Oils and Protozoa**

Secondary plant extracts, when fed to ruminants, decreased ammonia and methane production by decreasing the number of protozoa (Busquet et al., 2005b; Busquet et al., 2006; Lila et al., 2003; McGuffey et al., 2001; Wallace et al., 1994; Wang

et al., 1998). Defaunation is thought to reduce bacterial protein breakdown and re-synthesis in the rumen, therefore, increasing the flow of protein to the small intestine (Wang et al., 1998).

Protozoa differ from bacteria in the way they metabolize protein and in their feeding behavior; protozoa engulf particulate matter (bacteria, fungi and small feed particles) rather than attaching to feed. They ingest bacteria as their primary protein source (NRC, 2001). This contributes to N recycling in the rumen and may lead to reduced passage of microbial protein to the small intestine. Unlike most bacteria, protozoa cannot synthesize AA from ammonia. Because of this, protozoa are net exporters of ammonia so it is thought with defaunation ruminal ammonia concentrations will decrease (NRC, 2001). As discussed, secondary plant metabolites have been shown to decrease protozoa numbers and as such, may be a safe defaunating option (Lila et al., 2003; Wallace et al., 1994; Wang et al., 1998).

**Essential Oils and Methane Production**

Methane is produced in the rumen mainly by the bacteria *Methanosarcina* sp., *Methanomicrobium* sp., *Methanobrevibacter ruminantium*, *Methanobrevibacter* sp., and *Methanobacterium formicium* (Stewart, 1991). These bacteria form methane from $CO_2$ and $H_2$ and most of them can also utilize formate (Van Nevel and Demeyer, 1996). Methane production within the rumen can be beneficial in that the great affinity of methane bacteria for hydrogen keeps the partial pressure of hydrogen in rumen contents low. This helps to decrease the formation of lactate and ethanol (Van Nevel and Demeyer, 1996). Also, when *Ruminicoccus albus* is in the presence of a methanogen, more acetate is produced. This is coupled to generate more ATP by substrate level

phosphorylation (Wolin and Miller, 1988) which leads to increased efficiency of bacterial growth (Van Nevel and Demeyer, 1996).

With the benefits of increased methane production come drawbacks. An animal removes methane from its gastrointestinal tract by eructation, or respiration. This represents a loss of approximately 2-15% of the gross energy in the feed (Johnson et al., 1991; Holter and Young, 1992). For this reason, and because methane contributes to global warming (Beauchemin and McGinn, 2006; Crutzen et al., 1986; Moss, 1993; Varel and Miller, 2001), researchers are searching for ways to decrease methane production by ruminants. Secondary plant metabolites have been shown to decrease methane production in ruminant animals (Busquet et al., 2005b; Busquet et al., 2006; Lila et al., 2003; McGuffey et al., 2001). Busquet et al. (2005b) observed that garlic oil, when added to rumen fluid from animals fed a 50:50 alfalfa hay: concentrate diet, decreased methane production by 73.6%. This represented a reduction in methane production 30% greater than that observed with monensin.

**Essential Oils and Odor Control**

The odor emitted from cattle waste is the result of incomplete degradation of carbohydrate, protein and lipid components (Mackie et al., 1998; Varel et al., 1999). This incomplete degradation leads to the formation of short-chain VFA, aromatic chemicals, amines and other nitrogenous compounds, and sulfur-containing compounds. Complete degradation of waste leads to methane and carbon dioxide (Varel and Miller, 2001). Anoxic digesters for the production of methane were once popular in the 1970s and 1980s; however, the cost and expertise to operate them diminished their popularity

(Morse et al., 1996). Oxic treatment is just as expensive and does little to conserve nutrients (Varel and Miller, 2001).

Because of the antimicrobial properties of EO, they may be useful to decrease gaseous emissions from stored cattle waste. Varel and Miller (2001) conducted a study to determine if carvacrol and thymol would decrease gas and short-chain VFA production in stored cattle waste. The results indicated that a combination of the two EO will stop most fermentation activity in stored cattle waste. It was also noted that the combination of the two oils provided better antimicrobial action than a higher content of carvacrol or thymol alone (Manou et al., 1996; Paster et al., 1995). Varel and Miller (2001) concluded that carvacrol or thymol could be used as an additive to stored cattle waste to reduce odor-emissions, global-warming gases, and pathogens. This should retain nutrients or organic matter in the waste and enhance the fertilizer value. In addition, crops obtained from land fertilized with treated waste are likely to carry fewer food-borne pathogens than crops obtained from land fertilized with untreated waste (Varel and Miller, 2001).

## Differing Diets and Essential Oils

Different diets cause different proteolytic activities in the rumen because of their support of different bacterial communities (Newbold et al., 2004). For this reason, the composition of the diet that is fed seems to play a role in the effectiveness of secondary plant extracts on ruminal fermentation. The majority of the studies reviewed used diets that were comprised mainly of forage (50% or more) (Busquet et al., 2005a; Busquet et al., 2005b; Busquet et al., 2006; Cardozo et al., 2004; Castillejos et al., 2006; Lila et al., 2003; McIntosh et al., 2003; Newbold et al., 2004). The forages used were alfalfa hay,

sudangrass silage, grass and grass silage. Urea was not included in the diets. The authors of the aforementioned studies reported positive effects of EO on rumen fermentation. The positive effects of EO on rumen fermentation have included: increased propionate production (Busquet et al., 2005a; Busqet et al., 2006; Lila et al., 2003), decreased methane production (Busquet et al., 2005a; Busquet et al., 2005b; Busquet et al., 2006; Lila et al., 2003), decreased rumen ammonia N concentrations (Cardozo et al., 2004; Castillejos et al., 2006; Lila et al., 2003; McIntosh et al., 2003), and decreased deamination of AA by rumen bacteria (Cardozo et al., 2004; Castillejos et al., 2006; Newbold et al., 2004).

Beauchemin et al. (2006) fed a diet containing 75% barley silage and 0.59% urea in the concentrate mix to 16 Angus heifers and determined that EO had no effect on dry matter intake, ruminal fermentation parameters (VFA and pH), and methane emissions.

Cardozo et al. (2005) examined the effects of EO in a 10:90 straw:concentrate diet fed to beef steers. It was determined that at low ruminal pH (5.5) oregano, garlic, capsicum, yucca extracts and cinnamaldehye are potentially useful in beef diets because EO decreased ammonia N concentrations. It seems as though the effects of plant extracts on ammonia N concentrations are pH-dependent (Cardozo et al., 2005).

Hristov et al. (1999) investigated the effects of *Yucca schidigera* supplementation in a 39% alfalfa silage and 61% barley grain diet fed to Angus heifers on ruminal fermentation. *Yucca schidigera* decreased ammonia N concentrations and increased propionate concentrations in the rumen. The authors concluded that including the yucca plant may improve ammonia utilization in the rumen, most likely due to the partial

elimination of ruminal protozoa; therefore, enhancing microbial protein flow to the small intestine.

No studies could be identified in which EO supplementation was evaluated when corn silage was the main forage component of the diet. Only one study was found (Beauchemin et al., 2006) which used urea as a supplemental source of RDP.

## Conclusions

Future research should focus on the effects of EO supplementation in ruminants fed varying types of diets to determine which diets should be supplemented. The type of diet affects the microbial population in the rumen, and therefore, when EO are added they may or may not have an effect, depending on the microbial population. Research should also focus on adaptation of ruminal microorganisms to EO and combinations of EO. With the current increased awareness of antibiotic resistance, and the ban of antibiotic use in animal agriculture in Europe, and the increased number of organic farms, natural product use is on the rise. Research needs to focus on the mode of action within the rumen and their economic benefit of feeding EO.

# CHAPTER II

## ESSENTIAL OIL SUPPLEMENTATION OF A CORN SILAGE BASED DIET DEFICIENT IN RUMEN UNDEGRADABLE PROTEIN FED TO LACTATING HOLSTEIN DAIRY COWS

### Introduction

Plants have been seen by some for centuries as valuable resources, not only for consumption, but also for medicinal, preservative and antiseptic uses. The ruminant industry has taken notice of the antimicrobial properties of certain plant extracts and numerous in vitro and in vivo experiments (Busquet et al., 2005; Busquet et al., 2005a; Busquet et al., 2006; Cardozo et al., 2004; Cardozo et al., 2005; McIntosh et al., 2003; Newbold et al., 2004; Wallace, et al., 2004; and Wang et al., 1998) have been conducted to determine if some can be identified that promote a more efficient rumen fermentation and more efficient conversion of feed to animal product.

An increasing number of producers are looking for "natural products" to replace the use of antibiotic feed additives. "Natural" products include probiotics, prebiotics, enzymes, organic acids and secondary plant compounds (Wallace, 2004). The interest is in the secondary plant products, specifically essential oils (**EO**). Scientists across the globe have taken great notice of EO because of their proven benefits to rumen fermentation (Busquet et al., 2005a; Cardozo et al., 2004; Hristov et al., 1999; McIntosh et al., 2003; Newbold et al., 2004; Wallace et al., 2004).

Essential oils are steam-volatile, or organic-solvent, extracts of plants (Wallace, 2004). They give a plant its fragrance and are used by man for flavor, antiseptics and preservatives (Cowan, 1999; Wallace, 2004). They differ from fatty acids in that they contain extensive branching and are cyclized (Cowan, 1999) and are not catabolized for energy. They contain mainly hydrocarbons and alcohol, aldehyde or ester derivatives (Wallace, 2004).

Essential oils have been shown to have inhibitory effects against pathogenic bacteria and be beneficial to human health (Elgayyar et al., 2001; Imai et al., 2001, Marino et al., 2001 Shapiro et al., 1994). Oregano oil (Elgayyar, 2001), peppermint oil (Imai, 2001) and EO from other herbs (Marino, 2001) have all shown inhibitory effects on *Escherichia coli* O157:H7. Oils from cinnamon have been shown to inhibit *E. coli*, *Enterococcus faecalis*, *Staphylococcus aureus* (including the *S. aureus* resistant to methicillin), and *Salmonella* sp. (Chang, 2001). Essentail oils such as thymol and eugenol are potent against a wide range of oral bacteria and are therefore found in many antiseptic mouthwashes (Shapiro, 1994).

Nagy and Tengerdy (1968) determined that EO were not necessarily toxic to ruminal bacteria. In fact, studies following that work demonstrated that EO are beneficial to rumen fermentation (Busquet et al., 2005a; Cardozo et al., 2004; Hristov et al., 1999; McIntosh et al., 2003; Newbold et al., 2004; Wallace et al., 2004). Since the ban of growth-promoting antibiotic use in animal feeds in Europe at the end of 2005 (Wallace, 2004), researchers and scientists in the European dairy industry have shown great interest in alternatives to antibiotics. Currently, EO are being fed as an alternative to growth-promoting antibiotics in Europe (Wallace et al., 2002). It is their hope to identify EO that

will benefit rumen fermentation and feed efficiency in much the same way as growth-promoting antibiotics, such as ionophores, do.

VERTAN is the product used in this experiment and was provided by IDENA, Canada, Inc. VERTAN is a specific blend of thymol, eugenol, vanillin and limonene (IDENA Canada Inc., Ontario, Canada). The product was designed specifically for dairy cattle to improve ration palatability, achieve higher feed intake, and provide trace elements. Similar products, such as Crina Ruminant (Akzo Nobel Surface Chemistry Ltd., Hertfordshire, United Kingdom) have been shown to decrease ammonia production in the rumen (McIntosh, 2003). The authors speculated that EO inhibited the rate of deamination of AA in the rumen. Newbold (2004) reported a decrease in deamination of AA in vitro in rumen fluid removed from sheep supplemented with Crina at a rate of 110 mg/d, but saw no other effects of EO on rumen fermentation.

In most of the studies discussed above, the animals were fed a grass silage based diet which is commonly fed in Europe and Canada. However, in the United States, corn silage is a common feed for dairy cows because of the high yield per acre, the ease of storage, and the amount of digestible nutrients provided by corn silage. Therefore, the purpose of this study was to determine the effects of a specific blend of EO, in the form of VERTAN, on production parameters and rumen metabolism with cows fed a corn silage based diet.

## Materials and Methods

### Experimental Design and Treatments

Thirty multiparous Holstein cows were assigned to one of two treatments in a randomized complete block design. Cows were blocked according to calving date and assigned treatment at calving using a random number table. Dietary treatments were control (no VERTAN) and 0.08% VERTAN in diet DM. VERTAN supplementation ranged from 8.8 g/d to 23.4 g/d with the average amount throughout the trial being 20 g/d for the VERTAN containing treatment. Treatments were given 21 DIM and continued through 105 DIM. VERTAN was weighed out with Smartamine M and soybean meal (**SBM**). VERTAN, Smartamine M and SBM were weighed separately in the laboratory and then combined and mixed thoroughly. Soybean meal and Smartamine M was used as a carrier for VERTAN which was then top dressed onto the total mixed ration (**TMR**). There was additional SBM already present in the TMR. Smartamine M was included at a rate of 0.06% of diet DM and SBM (in the top-dress mixture) was included at a rate of 0.41% of diet DM. Fifteen cows received the Smartamine M/SBM top dress mixture and 15 cows received the SmartamineM/SBM/VERTAN mixture. The mixtures were top dressed and manually incorporated into the TMR at each feeding. All cows received the same pretreatment diet (Table 1) day zero to 20 postpartum. From day 21 to 105 all cows were fed the treatment diet (Table 1) formulated to be deficient in RUP and in excess of RDP according to NRC, 2001 (Table 3). Smartamine M was added to the treatment diet to balance for Met since RUP, and hence MP, was deficient in Met concentrations relative to requirements (NRC, 2001).

Two additional Holstein cows, previously fitted with ruminal cannulae, were assigned to one of the two dietary treatments in a switchback design. The cannulated cows were fed the treatment diet (Table 1) for four weeks; 3 weeks were used for an adjustment period and the fourth week samples were collected to determine ruminal ammonia N and pH. The cannulated cows were assigned treatment according to a random number table and then switched 3 times so that each cow had each treatment twice.

**Management of Cows**

All procedures related to animal care were conducted with the approval of the University of New Hampshire Institutional Animal Care and use Committee (Appendix A). Cows were housed in a naturally ventilated tie-stall barn and fed individually from feed tubs with free access to clean, fresh water. Cows were milked and fed three times daily at 8-h intervals (0500, 1300, and 2100h). Cows were milked in a milking parlor equipped with automatic take-offs and milk meters. Milk weights were recorded at each milking. Weather permitting, cows were provided daily exercise for 15-20 min before the 1300 h milking. Cows were weighed (Northeast Scale Co., Inc., Model: 708-5, Cardinal Scale Manufacturing Co.) twice a week, on Tuesday and Thursday, during the week prior to the experiment and then once a week, on Tuesday, until 105 DIM. Body condition scores (scale 1-5, with 1 being emaciated and 5 being obese; Wildman et al., 1982) were evaluated by five independent scorers and averaged across scorers to obtain one score. The two cannulated cows did not have their milk weights recorded, and were not weighed or BCS.

Feed intakes were measured daily with DMI determined once a week to allow for adjustment of dietary treatment amounts. Cows were fed in individual feed tubs that were closed before feeding and remained closed until all treatments were manually mixed into the TMR and all cows had returned from the milking parlor.

The diets (Tables 1) were fed as a TMR and prepared by weighing each ingredient and mixing in a mobile paddle mixer (Data Ranger; American Calan, Inc. Northwood, NH). Cows were fed 20% of their total daily feed allotment at 0500 h, 50% at 1300 h, and 30% at 2100 h for ad libitum feed intake. Diets were mixed using fresh feed before each feeding. Feed offered was adjusted daily to achieve 5-10% orts. Orts were collected and weighed daily at 1100 h. Orts were examined regularly to ensure complete consumption of the test product. Dry matter intakes were calculated daily.

**Feed Sampling and Analysis**

All feed ingredients were sampled 14-d before the start of the experiment and analyzed for CP, NDF, ADF, and $NE_L$ to assist in determining the final formulation of the diets. Thereafter, silages were sampled daily during the a.m. feeding and analyzed for DM. Total mixed ration samples were taken at each feeding each day from Sunday noon through Friday a.m. and composited for DM analysis and particle separation. Ort samples were collected at 1100 h Monday through Friday and composited for DM analysis and particle separation. Each month, concentrate feeds and silages were sampled on two consecutive days, composited as collected and sent for analysis of CP, NDF, ADF, and $NE_L$ (Dairy One, DHI Forage Testing Laboratory, Ithaca, NY) for ration adjustment. Concentrate feeds and silages were sampled one day per week. Samples were dried to approximately 90% DM in a forced air oven at 60°C (VWR Scientific, NJ)

allowed to equilibrate to room temperature, and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Ground samples were composited bi-monthly, based on the number of cows per week. Samples were analyzed for DM, CP, soluble protein, NDF, ADF, $NE_L$, ADICP, NSC, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S (Dairy One, DHI Forage Testing Laboratory, Ithaca, NY) (Table 2) to assist in determining the final formulation of the diets.

**Evaluation of the Pretreatment and Treatment Diets**

The pretreament and treatment diets were evaluated twice a week for particle size, using the Penn State Shaker (Henrichs, 1996), and DM. All required animal and feed data were entered into the NRC (2001) model to determine the chemical composition of the pretreatment and treatment diets and their nutritional adequacy. Chemical composition of each feed was entered into the model. Actual average lactation number, DMI, DIM, days pregnant, BW, BCS, milk yield, and milk components were inputted. Default values were used for age at first calving, calving interval, and calf birth weight (Table 3).

**Milk Sampling and Analysis**

Milk samples were obtained from each cow during all three milkings one day each week through 105 DIM. The first milk sample was taken 15 DIM for two consecutive days and then once a week thereafter. Samples were refrigerated until composited by milk weight for the sample date. Samples were preserved with 2-bromo-2nitropropane-1,3-diol (1 tablet per 40ml of milk). Samples were analyzed for crude protein, true protein, fat, lactose, and milk urea N (**MUN**) using infrared (DairyOne Milk

Laboratories, Ithaca, NY) by using a Foss Milkoscan 4000 (Foss Electric, Hillerød, Denmark).

**Blood Sampling and Analysis**

Blood samples were taken by venipuncture of the coccygeal vein at 0900 h every Tuesday and Thursday. Blood was collected in 10-ml evacuated tubes (Vacutainer®, Becton Dickinson, Rutherford, NJ) containing EDTA using 20x1 gauge needles. Blood tubes were immediately placed into an ice bath and centrifuged within 45 min at 3300 x g for 20 min at 5°C (IEC, Needham Heights, MA). A 2-ml aliquot from each sample was placed in a labeled 5 ml polypropylene tube and stored at -20°C until analyzed for blood urea N (BUN). Blood urea N concentrations were determined using Procedure No. 535 of Sigma Chemical Co. (Sigma Chemical Co., St. Louis, MO).

**Rumen Sampling and Ammonia N and pH Analysis**

Twenty four rumen fluid samples were collected representing every hour of a 24-h day from the two ruminally cannulated cows. Rumen fluid was collected using a manual vacuum applied through 1.27-cm polyvinyl chloride pipe and inserted into a slit in the rumen cannula stopper. Approximately 500 ml of fluid was collected from at least three sites in the rumen (front, middle, and back) approximately 48 cm deep. The rumen fluid was mixed manually, and the pH of the fluid was determined immediately using a portable pH meter (Orion model 230A, pH triode electrode; Orion Research, Inc., Boston, MA). The rumen fluid was strained through 2 layers of cheesecloth. Two 40 ml strained rumen fluid samples were added to 50 ml polypropylene centrifuge tubes containing 2.4 ml of 0.6N HCl for $NH_3$ analysis. Excess rumen fluid was discarded. The $NH_3$ samples were frozen and stored at -20°C until analyzed. Analysis for $NH_3$ was

conducted using an Orion Specific Ion Meter with a gas-ammonium electrode (Orion model 407A meter with 95-12 electrode; Orion Research, Inc., Boston, MA) (Schwab et al.,1992).

**Statistical Analysis for the Lactation Experiment**

Production data was analyzed using the MIXED procedure of SAS® (release 9.1) according to the following model:

$$Y_{ijk} = \mu\ \mu + B_i + O_j + \beta X_{ij} + W_k + PW_{jk} + E_{ijk}$$

Where:

$Y_{ijk}$ is the dependent, continuous variable,

$\mu$ is the overall mean,

$B_i$ is the random effect or the block (i = 1,....15)

$O_j$ is the fixed effect of the EO (j = 1, 2),

$\beta$ is the regression (covariate) coefficient,

$X_{ij}$ is the covariate measurement,

$W_k$ is the fixed effect of week of experiment (k = 1,...,12),

$E_{ijk}$ is the residual errors

In this model, the random effect of cows within treatment subclasses was used as the error term for the effect of the VERTAN. Residual errors, which are errors within cows across time and represent errors from repeated measurements in the experimental units (cows), was modeled using a first-order autoregressive covariance structure. Degrees of freedom were calculated using the Kenward-Roger option of MIXED procedure (SAS®, 2001). A covariate term was included in the model to reduce the variance due to cows within treatment subclasses. The covariate variables were taken

from 15 DIM to 21 DIM and consist of milk production, milk composition, DMI, BW, BCS, and BUN as appropriate. The DIFF option in SAS® was used to test treatment differences among least squares means. The SLICE option in SAS® was used to analyze differences among weekly treatment means. Significance was declared at $P < 0.05$.

**Statistical Analysis for the Rumen Metabolism Experiment**

Rumen data was analyzed using the MIXED procedure of SAS® (release 9.1) according to the following model:

$Y_{ijk} = \mu + O_i + P_j + c(S)_{ij} + E_{ijk}$

Where:

$Y_{ijk}$ is the dependent, continuous variable,

$\mu$ is the overall mean,

$O_i$ is the fixed effect of the essential oils (i = 1, 2),

$P_j$ =is the random effect of the $j^{th}$ period (j = 1,2)

$c(S)_{ik}$ is the random effect of the $k^{th}$ cow with the $i^{th}$ treatment of essential oils (k= 1,....6)

$E_{ijk}$ is the residual errors

Fixed effects include treatment while the random effects are cow (treatment) and period. Least square means were determined and significance was declared at $P< 0.05$.

**Results**

The average ingredient compositions of the pretreatment and treatment diets are presented in Table 1. Both diets were changed 9 times throughout the 255-d trial because of changes in the chemical composition of the feeds. The values for the diets were arrived at by calculating the number of cow days per diet and calculating an average

amount of each ingredient used based on the number of cows it was fed to. The reason for the reported low content of grass hay in the treatment diet is because it was fed only 12% of the cow days and at 0.9% of diet DM during the time period it was in the diet.

The chemical composition of the feeds is presented in Table 2, and Table 3 lists the NRC (2001) evaluation of the diets. Rumen undegradable protein was deficient in both diets, while RDP was in a slight surplus; therefore, MP was deficient in both diets. Energy was more limiting in the pretreatment diet than in the treatment diet (Table 3).

Dry matter intake was not affected by VERTAN supplementation (Table 4). Intakes of DM averaged 22 kg/d during wk 4 and steadily increased to 25 kg/d during wk 15 (Fig. 1). There was no response to treatment in milk yield (Table 4). Milk yield averaged 47 kg/d during wk 4 and was generally maintained at that level through wk 15 with a slight increase at wk 8 and 9 (Fig. 1). Milk components were not significantly different among treatments (Table 4). Milk and blood urea N concentrations were not affected by EO supplementation (Tables 4 and 5). Milk urea N averaged 11 mg/dl during wk 4 with slight variance through wk 15 (Fig. 2). Blood urea N levels averaged 25 mg/ml during wk 4 with slight variance through wk 15 (Fig. 2). Feed efficiencies also did not differ between control and VERTAN supplemented cows (Table 4).

There was no difference in average BW or BCS between treatments (Table 5). There also was no difference in BW or BCS changes between treatments throughout the 15-wk treatment period (Fig 1). Body weights averaged 665 kg at wk 4 and increased slightly through wk 15. Body condition scores averaged 3.0 at wk 4, decreased slightly through wk 8, and then were maintained at an average of 2.9 through wk 15 (Fig. 1).

VERTAN supplementation had no affect on rumen pH in the rumen cannulated cows (Table 5); pH was maintained at an average of 6.0 through 24-h (Fig. 3). VERTAN supplementation on rumen ammonia N concentrations over a 24-h period had a significant hour by treatment affect (Table 5). VERTAN appeared to be most effective when ammonia N concentrations were the highest in the rumen, and more so after the noon and evening feedings (Fig.3).

## Discussion

### Diets and Treatments

Previous research (Cardozo et al., 2004; Castillejos et al., 2006; Newbold et al., 2004) demonstrated that EO inhibit deamination of AA by ruminal bacteria. In the current study, the diet was formulated to be deficient in RUP, contain a surplus of RDP relative to requirements, and contain adequate energy to meet requirements (NRC, 2001). This was done to help ensure that if AA deamination was inhibited by VERTAN supplementation, a greater proportion of the AA supplied by RDP would pass to the small intestine for absorption and RUP supplies would not be limiting. This could be beneficial due to the high cost of RUP supplementation. Also, because fewer AA would be deaminated in the rumen, lower ammonia concentrations in the rumen were expected with VERTAN supplementation.

Formulating the diet to meet the energy requirements of all animals was challenging. It was difficult because the cows were assigned to the trial at calving, and the average DIM was used in making adjustments to the diet throughout the trial. As a consequence, the diet actually fed to the cows was deficient in energy. However, the cows maintained a normal DMI and milk yield curve (NRC, 2001). Body weight

changes and BCS were adequate for early lactation cows as well (NRC, 2001). Because the cows did not lose weight or drop dramatically in BCS, it did not seem as though excessive body reserves were mobilized for milk production.

Forage quality was less than optimal, and there were frequent changes in forage quality throughout the trial. According to NRC (2001) the majority of the time the forages being fed were mature. This made it difficult to meet the energy needs of the animal while maintaining a high forage diet that contained an excess of RDP. Therefore, urea was added to formulate the diet to contain excess RDP.

Corn silage is a common forage source in the U.S. because of the high yield per acre and the amount of digestible nutrients it provides. It is common to feed grass silage based diets in Europe and Canada (Newbold et al., 2004; McIntosh et al., 2003). Benchaar et al. (2003) investigated the effects of 0.75 g/d of EO supplementation in a corn silage vs. a grass silage based diet on nutrient digestibility, N retention, duodenal bacterial N flow, and milk production and composition in lactating dairy cows. No interaction was observed between EO and silage source and EO supplementation to either diet had no effect on nutrient digestibility, duodenal bacterial N flow and milk production and composition. This study suggests that EO are not diet specific and more investigation is needed to evaluate the effectiveness of different types of EO and different dose levels on the performance of dairy cows.

**Lactation Trial**

In the current study, it was expected that cows supplemented with 0.08% VERTAN in diet DM would have higher milk yield (because of increased lactose synthesis) and increase concentrations of milk fat and true protein compared to control

cows. Previous studies (Busquet et al., 2005a; Cardozo et al., 2004; Castillejos et al., 2004) indicated that the VFA profile of cows supplemented with EO is altered in such a way that yields of milk fat and lactose could increase, similar to that of ionophore supplementation. An increase in milk protein concentrations are expected when microbial protein provides a greater proportion of MP supplies. This is expected in part because ruminally synthesized microbial protein has a better AA balance than RUP. The treatment diet was formulated to contain a surplus of RDP and be deficient in RUP and hence, in MP supplies. Thus, any partitioning of RDP to RUP by VERTAN would have the net effect of increasing the AA supply to the small intestine and reducing any deficiency in MP that might have existed.

Benchaar et al. (2003) examined the effects of supplementing 0.75 g/d of EO to lactating Holstein dairy cows on milk yield and composition. The cows were fed ad libitum twice daily. The cows received either corn silage or alfalfa silage based TMR with or without supplemental EO. There was no effect on DMI or milk production when EO were supplemented to either diet. Except for lactose, which was increased by EO, milk composition was not influenced by EO supplementation.

Daniels et al. (2006) investigated the effects of supplementing 0, 28 or 56 g/d of a blend of plant botanicals to 260 lactating Holstein cows on lactation performance. There was no effect of treatment on milk yield or milk protein content. Milk urea N was decreased by both levels of the treatment (28 g/d and 56 g/d). Milk fat percent was increased with 28 g/d of supplementation but was not different from control when the botanicals were supplemented at the higher level (56 g/d). Supplementing cows with 56 g/d of plant botanicals decreased DMI and milk fat yield; therefore, FCM yield

decreased. The authors determined that supplementing plant botanicals to lactating Holstein cows at a rate of 28 g/d was beneficial, while supplementing plant botanicals to lactating cows at a rate of 56 g/d had negative effects on production performance.

Most other work examining the benefits of supplementing EO to ruminant diets has been done in vitro or with other ruminant animals (Newbold et al., 2004) and focused on the effect of EO on ruminal fermentation (Busquet et al., 2005a; Busquet et al., 2005b; Busquet et al., 2006; Cardozo et al., 2005; Castillejos et al., 2006). Due to the limited number of studies that have examined the effects of EO on lactation performance it is difficult to determine why an effect of EO supplementation on milk yield and milk composition was not observed in the present study.

**Rumen Metabolism Trial**

In the current study, it was expected that ammonia N concentrations would be decreased with VERTAN supplementation because the treatment diet was formulated in such a way (high RDP and low RUP) that any decreased deamination of AA to ammonia would have been seen between treatments.

Ammonia N Concentrations. Beauchemin and McGinn (2006) fed 16 Angus heifers a 75% barley silage diet supplemented with 0.59% urea. Treatments were no additive (control), 175 g/d of fumaric acid, 1 g/d of EO (Crina Ruminants), or 4.6% of DMI of canola oil for four 21-d periods in a replicated 4 x 4 Latin square design. At the start of the experiment the heifers were approximately 8 mo of age and weighed 260 $\pm$ 32 kg. Essential oils had no effect on DMI, average daily gain, or on ruminal fermentation variables (VFA concentrations, rumen ammonia N concentration and pH).

Newbold et al. (2004) fed 4 mature sheep a 60:40 grass silage:concentrate diet with no supplemental urea. The sheep were supplemented with 0.11 g/d of EO. Essential oils had no effect on ruminal pH or ammonia N concentrations. Rumen fluid was sampled 2-h after feeding and was used to determine the proteolytic, peptidolytic and deaminative activities of rumen microorganisms. Proteinase activities were measured by the methods described by Wallace (1983) using the release label from [$^{14}$C] labeled casein. Peptidase activity was measured with alanine peptides as substrates (Wallace and McKain, 1989) and deaminase activity was determined with casein acid hydrolysate as the substrate (Newbold et al., 1990). The investigators concluded that EO supplementation had no effect on the degradation of $^{14}$C labeled casein, dialanine or penta-alanine, but deamination of AA was inhibited by 24%. Microbial protein synthesis, however, was not affected.

Decreased ammonia N concentrations were observed in vitro (Castillejos et al., 2006; Lila et al., 2003) as well as in vivo (McIntosh et al., 2003) with EO supplementation. In the current study, a significant hour by treatment effect was seen in cows supplemented with VERTAN on ruminal ammonia N concentrations (Fig3). It seems as though VERTAN decreased ruminal ammonia N concentrations when concentrations in the rumen were high, right after feeding. The decrease was more prominent after the afternoon and evening feedings. With the limited studies done in vivo looking at a 24-h feeding period, it is hard to say why this is. Also, it is possible that had we used more than two cannulated cows for this experiment we would have seen an effect throughout the 24-h period.

In the present study, ruminal free AA concentrations were not measured. However, if the ruminal free AA concentrations were determined, an increase in ruminal free AA concentrations may have been observed in the rumen fluid of the VERTAN supplemented cows indicating a reduction in the deamination of AA supplied by RDP by rumen microbes because a significant decrease in ruminal ammonia N concentrations was observed.

Essential Oils and Ionophores. Ionophores, such as monensin and lasalocid, are fed to cattle to reduce the age at puberty (Meinert et al., 1992), decrease methane production (McGinn et al., 2004), increase propionate concentrations in the rumen (Chen and Wolin, 1979), decrease ammonia production, and decrease the acetate to propionate ratio (Russell and Strobel, 1989). Quite often ionophores are added in experiments investigating plant extracts to compare the effects (Benchaar et al., 2004; Buquet et al., 2005a; Castillejos et al., 2006; McGinn et al., 2004).

Benchaar et al. (2004) supplemented 0.22 g/d of monensin or 2 or 4 g/d of EO to 20 steers and 20 heifers (Angus x Hereford). Initial BW was 369 $\pm$ 29 kg. The cattle were fed a TMR comprised of 75% grass/legume silage and 25% rolled barley. The experiment lasted 98 and 82 days for the steers and heifers, respectively. Dry matter intake was not affected by the addition of EO at 2 or 4 g/d but was 10% lower in animals supplemented with monensin. Average daily gain was not affected by either of the two supplements. Feed efficiency was not different between control animals and those receiving treatment; however, animals receiving 2 g/d of EO had an improved feed efficiency over those supplemented with 4 g/d.

Castillejos et al. (2006) used 8 dual-flow continuous culture fermenters to examine the effect of thymol and eugenol on rumen fermentation. Monensin was included as a positive control. The treatments were 10 g/d of monensin, 5, 50 or 500 mg/d of thymol and 5, 50, and 500 mg/d of eugenol. The fermenters were fed 95 g/d of DM of a diet formulated to meet requirements for lactating dairy cows (18.0% CP, 30.2% NDF, 21.7% ADF; NRC 2001). The diet consisted of 60% alfalfa hay and 40% concentrate. As expected, monensin maintained total VFA concentrations while increasing the proportion of propionate and reducing the proportion of acetate and butyrate in ruminal fluid. The 500 mg/d thymol treatment reduced total VFA production, while 50 mg/d thymol had no effect on VFA profile. However, 5 mg/d thymol tended to reduce the proportion of acetate and increase the proportion of butyrate without affecting total VFA concentrations. The 500 mg/d eugenol treatment reduced total VFA concentration, the proportion of acetate, and the acetate to propionate ratio.

Benchaar et al. (2004) and Castillejos et al. (2006) suggested that ionophores and EO have similar effects in the rumen, and EO may be able to replace ionophores as a safe way to manipulate the rumen. But the authors also suggested that further research is warranted to determine the most effective dosage of each EO.

Dose Levels. Castillejos et al. (2006) conducted an in vitro experiment to evaluate the effect of supplementing 5 EO compounds (thymol, eugenol, limonene, guajacol, and vanillin) in 5 doses (5, 50, 500, and 5000 mg/L) to in vitro batch cultures. The authors concluded that the effect of each of the EO compounds on rumen microbial activity differed depending on the dose. At the 5000 mg/L dose, all the EO decreased VFA concentrations. Limonene supplemented at 500 mg/L decreased ammonia N

concentrations, indicating that deamination of AA was inhibited. Guajacol reduced the proportion of acetate and ammonia N at 500 mg/L, but had negative effects (reduced VFA concentrations) on rumen fermentation at 5 and 50 mg/L. It is not clear why these effects were observed. The authors suggested that vanillin is not a good alternative to improve rumen microbial fermentation because no effects of supplementation were observed. The optimum dose for thymol was difficult to determine because both positive effects (changes in the profile of VFA) and negative effects (reduction in total VFA concentration) were observed. Eugenol supplemented at 500 mg/L reduced ammonia N concentrations in the media.

In the current study, VERTAN (a specific blend of thymol, eugenol, vanillin and limonene) was supplemented at an average rate of 20 g/d. As just discussed, Castillejos (2006) reported that limonene and eugenol supplemented at 500 mg/L (estimated to be 75 g/d) were beneficial to ruminal fermentation, but the optimal concentrations of thymol and vanillin in the rumen were more difficult to define. The observations of Castillejos et al. (2006) may indicate that the rate of VERTAN supplementation could be increased to have greater effect on ruminal ammonia N concentrations. Also, the types of EO in VERTAN could be manipulated to increase its effect, such as removing vanillin since it may have no effect in the rumen and adding more of another EO that has been shown to benefit rumen fermentation.

Beauchemin and McGinn (2006) found no effect of supplementing CRINA (Crina Ruminant; Akzo Nobel Surface Chemistry Ltd., Hertfordshire, United Kingdom) to Angus heifers at a rate of 1 g/d on DMI, ADG, or on ruminal fermentation variables (VFA, ammonia-N concentration and pH). The mixture of EO in CRINA is similar to

that of VERTAN. The amount of EO supplemented to the heifers may have been insufficient to observe an effect.

Hristov et al. (1999) determined that feeding 20 or 60 g/d of *Yucca schidigera* (a plant high in steroidal glycosides known as saponins) to 6 Angus heifers resulted in decreased rumen ammonia N concentrations and increased ruminal propionate concentrations at both dose levels. Ruminal VFA concentrations were not determined in the present study. A study to follow should examine the optimum dose level of VERTAN on rumen ammonia N and VFA concentrations.

Busquet et al. (2006) and Castillejos et al. (2006) noted that the amount of EO supplemented that maximize the benefit to ruminal fermentation needs to be further examined. In addition, providing the mixtures of EO that work best in the rumen environment should also be considered (Busquet et al., 2006; Lila et al., 2003; Varel and Miller, 2001).

Rumen pH. The average particle separations for the TMR fed during the treatment period for the top, middle, and bottom screens were 16 $\pm$ 5, 34 $\pm$ 5, 50 $\pm$ 5%, respectively. The particle separation of the orts from control cows and cows supplemented with VERTAN averaged 26 $\pm$ 11, 34 $\pm$ 7, 39 $\pm$ 9% (top, middle, bottom) and 32 $\pm$ 12, 34 $\pm$ 8, 35 $\pm$ 7% (top, middle, bottom), respectively. Orts averaged 11 $\pm$ 10 % of feed offered.

The particle separation analysis suggests that the cows supplemented with VERTAN were sorting out smaller feed particles because a more than optimal amount remained on the top screen of the orts of the VERTAN supplemented cows. Cows not receiving the proper amount of long forage particles will most likely drop in rumen pH

level and, therefore, be more prone to rumen acidosis (Stone, 2004). pH levels in the VERTAN and control cows ranged from 5.4 to 6.7 and 5.5 to 6.5, respectively. These values were determined by obtaining approximately 500 ml of rumen fluid from at least three sites in the rumen (front, middle, and back) approximately 48 cm deep. The rumen fluid was mixed manually, and the pH of the fluid was determined about 5 minutes after the sample was taken, using a portable pH meter.

Subacute ruminal acidosis is defined at ruminal pH of approximately 5.2 to 5.6 (Owens et al., 1996, 1998). Cows most prone to subacute ruminal acidosis are those that are transition animals or high DMI animals, such as the cows in the current study. The method used to measure pH influences the pH measurement. pH measurements taken outside the rumen, immediately after collection (as done in the current study) gives recordings 0.1 units higher than measurement taken within the rumen (Stone, 2004). This suggests that the cows in the current study may have been prone to subacute rumen acidosis, which may have affected the efficacy of the EO in the rumen (Cardozo et al., 2005; Hristov et al., 1999).

Cardozo et al. (2005) obtained rumen fluid from 2 beef heifers fed a 10:90 straw:concentrate diet to determine the effects of 6 natural plant extracts and 3 purified secondary metabolites at controlled pH levels of 7.0 and 5.5 on in vitro rumen microbial fermentation. As expected, propionate concentrations increased at a lower pH while acetate concentrations decreased. Most fibrolytic ruminal bacteria are generally more sensitive to low pH levels than sacchralytic bacteria, and the fibrolytic bacteria produce greater amounts of acetate (Russell and Dombrowki, 1980; Hoover, 1986). The authors

also concluded that EO decreased ammonia N concentrations at the lower pH level and had no effect at pH of 7.0.

Hristov et al. (1999) observed positive effects (decreased ammonia and increased propionate concentrations) of *Yucca schidigera* supplementation on rumen fermentation with rumen pH higher than 6.1 when supplemented to Angus heifers fed a 39:61 alfalfa silage:concentrate diet.

In the current study, the range of ruminal pH for the 2 cannulated cows supplemented with VERTAN was 5.4 to 6.7. The average pH for each cow, regardless of treatment, was 5.9 and 6.1 throughout the experiment. It is interesting to note that when pH was lowest was when VERTAN seemed to have the most effect on ruminal ammonia N concentrations (Fig. 3). The cows were at a marginal risk for subacute ruminal acidosis because the NDF, forage NDF, average pH, and NFC were 31.6, 24.5, 6.0, and 43.4, respectively; all indicate marginal subacute ruminal acidosis (Stone, 2004).

De Veth and Kolver (2001) declared suboptimal pH at 5.4 and optimal pH at 6.3. They conducted an in vitro study to determine if variable periods of pH at 5.4 would reduce microbial protein synthesis and fiber digestion. They controlled the pH of rumen fluid at 5.4 for hours 0, 4, 8 and 12 and then brought it back to pH 6.3 each 24-h day. Results indicated that at pH 5.4, microbial N and dietary N, as a percent of non-ammonia N, were 51.5, 49.1, 42.8, 38.6 and 48.5, 51.0, 57.2, 61.5, respectively. Ammonia N concentrations were not affected by pH.

## CONCLUSIONS

Essential oils, in the form of VERTAN, supplemented to a corn silage based diet fed to Holstein dairy cows in early lactation had no effect on milk production or milk

composition, or on ruminal pH. However, VERTAN supplementation did result in a significant treatment by hour effect on ruminal ammonia N concentrations. Ammonia concentrations were decreased the most with VERTAN after feeding when ammonia concentrations are the highest. This work confirms the results of others who have demonstrated that EO suppress AA deamination and indicates that EO are likely effective in a corn silage based diet only when dietary RDP is in excess of requirements and dietary RUP is deficient. Research defining the best combination of EO and the proper levels of EO supplementation in a corn silage based diet fed to lactating cows is essential to future use of EO in many North American dairy production systems. In addition, research that identifies the effects of the interactions between diet type and EO supplementation on lactation performance is warranted.

Table 1. Ingredient composition of the total mixed rations.

| Ingredient, (% of DM) | Pretreatment | Treatment |
|---|---|---|
| Corn silage | 32.1 | 29.8 |
| Mixed, mostly grass silage | 14.0 | 14.9 |
| Legume hay | 7.7 | 7.2 |
| Grass hay | 0.53 | 0.11 |
| Corn, ground | 13.8 | 15.1 |
| Corn, steam flaked | 5.6 | 6.4 |
| Beet pulp | 1.8 | 1.6 |
| Citrus pulp | 1.8 | 1.6 |
| Soybean hulls | 3.6 | 4.3 |
| ProvAAl Elite™[1] | 2.1 | 0.0 |
| Molasses | 0.38 | 0.79 |
| Soybean meal | 10.5 | 11.9 |
| Urea | 0.34 | 0.43 |
| Smartamine M™ | 0.0 | 0.06 |
| Megalac | 1.9 | 2.5 |
| Vitamin premix[2] | 3.54 | 3.4 |

[1]Venture Milling Inc., 212 S. Bradford, Seaford, DE 19973. Product contained: bloodmeal, hydrolyzed feather meal, DL-methionine hydroxy analogue, DL-methionine, sodium bentonite, natural and artificial flavor, 0.825% poly(2-vinylpryridine co-styrene), 93.0% CP, 1.1% fat, 1.3% ash, Arg 4.8% of CP, His 5.4% of CP, Ile 1.2% of CP, Leu 12.6% of CP, Lys 8.8% of CP, Met 3.9% of CP, Phe 6.8% of CP, Thr 3.4% of CP, Trp 1.2% of CP, Val 6.3% of CP.

[2]Contained: 23.7 % sodium bicarbonate, 21.4% calcium carbonate, 14.0 % salt, 8.5% biophosphate, 8.2% potassium/magnesium sulfate, 7.7 % magnesium oxide(54%), 4.9% microlite, 3.9 % DF Yeast DFM, 3.4% potassium chloride, 1.4 % ZinPro®4 Flex (Zinpro Corp., Eden Prairie, MN), 0.9% MTB-100, 0.5% zinc sulfate, 0.5% mineral oil, 0.4% selenium-plex 2000, 0.4% manganese sulfate, 0.1% copper sulfate, 0.1% hay sugar ADE, 0.2% vitamin E (50 IU/kg), 0.04% vitamin A (65M IG/g), 0.008% vitamin $D_3$ (50M IG/g), 0.007% cobalt sulfate, 0.003% calcium iodate.

Table 2. Chemical composition of consumed feeds.

| Item, (% of DM) | Corn silage | MMG[1] silage | Legume hay | Grass hay | Corn grain | Corn grain, SF[2] | Soybean hulls | Beet pulp | Citrus pulp | Soybean meal |
|---|---|---|---|---|---|---|---|---|---|---|
| CP | 8.0 | 16.1 | 21.4 | 7.0 | 8.6 | 8.8 | 11.5 | 7.9 | 6.8 | 53.4 |
| ADF | 25.9 | 43.7 | 32.5 | 44.1 | 4.2 | 3.8 | 49.6 | 30.8 | 21.0 | 6.2 |
| NDF | 40.8 | 62.6 | 41.1 | 70.9 | 9.1 | 8.2 | 67.0 | 40.5 | 21.6 | 11.4 |
| Lignin | 3.2 | 5.9 | 7.5 | 6.7 | 1.1 | 1.4 | 2.7 | 2.5 | 1.4 | 0.7 |
| ADICP | 0.78 | 2.2 | | 0.8 | 0.8 | 0.9 | 1.4 | 1.6 | 1.6 | 0.3 |
| NDICP | 1.0 | 4.8 | | 2.5 | 0.9 | 1.2 | 4.5 | 5.5 | 2.4 | 2.4 |
| Fat | 3.2 | 4.7 | 2.2 | 2.0 | 4.2 | 4.2 | 1.9 | 0.7 | 2.5 | 1.8 |
| Ash | 3.8 | 9.29 | 9.8 | 6.03 | 1.43 | 1.43 | 4.94 | 7.7 | 4.36 | 6.85 |
| TDN | 72 | 57 | 61 | 54.5 | 88 | 88 | 64 | 68 | 79 | 81 |
| $NE_L$, Mcal/lb | 0.75 | 0.51 | 0.63 | 0.41 | 0.95 | 0.95 | 0.66 | 0.7 | 0.84 | 0.85 |
| Ca | 0.18 | 0.56 | 1.18 | 0.33 | 0.02 | 0.01 | 0.57 | 0.86 | 1.8 | 0.39 |
| P | 0.22 | 0.33 | 0.27 | 0.23 | 0.27 | 0.27 | 0.11 | 0.08 | 0.10 | 0.75 |
| Mg | 0.12 | 0.23 | 0.24 | 0.15 | 0.10 | 0.10 | 0.23 | 0.28 | 0.11 | 0.3 |
| K | 0.86 | 2.44 | 2.81 | 1.41 | 0.31 | 0.30 | 1.19 | 0.29 | 0.84 | 2.21 |
| S | 0.09 | 0.22 | 0.24 | 0.12 | 0.10 | 0.10 | 0.11 | 0.21 | 0.08 | 0.41 |
| Fe, ppm | 297 | 424 | 178 | 96 | 35 | 36 | 437 | 1010 | 73 | 104 |
| Zn, ppm | 22 | 31 | 24 | 18 | 21 | 20 | 39 | 20 | 10 | 46 |
| Cu, ppm | 6 | 11 | 9 | 8 | 2 | 2 | 7 | 8 | 6 | 15 |
| Mn, ppm | 19 | 77 | 46 | 38 | 7 | 6 | 14 | 82 | 7 | 34 |

[1]MMG = mixed, mostly grass.
[2]SF = steam-flaked.

Table 3. NRC (2001) evaluation of consumed diets.

| Item[1] | Pretreatment | Treatment |
|---|---|---|
| NDF, %DM | 32.3 | 31.6 |
| Forage NDF, %DM | 25.8 | 24.5 |
| ADF, %DM | 21.7 | 21.2 |
| NFC, %DM | 42.6 | 43.4 |
| ME, Mcal/kg DM | 2.58 | 2.53 |
| EE, %DM | 4.7 | 5.2 |
| DCAD, mEQ/kg | 145 | 319 |
| $NE_L$, Mcal/kg DM | 1.65 | 1.62 |
| | | |
| $NE_L$ required, Mcal/d | 42.0 | 40.5 |
| $NE_L$ supplied, Mcal/d | 31.8 | 37.6 |
| $NE_L$ balance, Mcal/d | -10.3 | -3.0 |
| MP required, g/d | 2571 | 2658 |
| MP supplied, g/d | 2208 | 2461 |
| MP balance, g/d | -363 | -196 |
| DM intake-actual, kg/d | 19.3 | 23.2 |
| DM intake-predicted, kg/d | 17.8 | 25.8 |
| | | |
| $NE_L$ allowable milk, kg/d | 29.0 | 42.2 |
| MP allowable milk, kg/d | 34.7 | 41.8 |
| Actual milk, kg/d | 43.0 | 46.8 |
| | | |
| CP, %DM | 17.3 | 16.6 |
| RDP, %DM | 10.8 | 10.9 |
| RUP, %DM | 6.5 | 5.6 |
| RDP balance, g/d | 135 | 219 |
| RUP balance, g/d | -433 | -235 |
| RDP balance, % | 107 | 109.5 |
| RUP balance, % | 74.5 | 84.7 |

[1] Values predicted from Table 1.

Table 4. Effect of feeding VERTAN to lactating Holstein cows on DMI, milk production, feed efficiency, and milk composition.

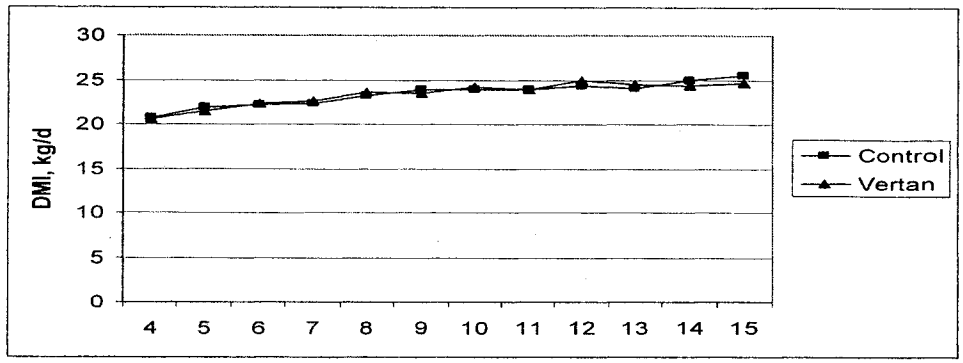
| | Treatment | | | *P*-value | |
|---|---|---|---|---|---|
| Item | Control | VERTAN | SE[1] | Treatment | Trt*Week |
| DM intake, kg/d | 23.4 | 23.3 | 0.27 | 0.93 | 0.70 |
| Milk yield, kg/d | 47.4 | 46.2 | 0.67 | 0.22 | 0.99 |
| 3.5% FCM[2] | 47.7 | 47.1 | 0.65 | 0.50 | 0.50 |
| ECM[3] | 44.0 | 43.7 | 0.99 | 0.85 | 0.59 |
| Milk/DMI | 2.02 | 1.99 | 0.02 | 0.42 | 0.53 |
| FCM/DMI | 1.47 | 1.44 | 0.02 | 0.37 | 0.50 |
| ECM/DMI | 1.14 | 1.12 | 0.02 | 0.52 | 0.65 |
| Milk components | | | | | |
| Fat, % | 3.55 | 3.61 | 0.04 | 0.28 | 0.13 |
| Fat yield, kg/d | 1.68 | 1.67 | 0.03 | 0.87 | 0.25 |
| True protein, % | 2.74 | 2.71 | 0.03 | 0.45 | 0.80 |
| True protein, kg/d | 1.29 | 1.26 | 0.02 | 0.22 | 0.91 |
| MUN, mg/dl | 11.2 | 10.9 | 0.19 | 0.33 | 0.62 |
| Lactose, % | 4.79 | 4.76 | 0.02 | 0.11 | 0.22 |
| Lactose yield, kg/d | 2.27 | 2.20 | 0.04 | 0.14 | 0.63 |
| SNF[4], % | 12.0 | 12.0 | 0.06 | 0.69 | 0.42 |
| SNF yield, kg/d | 5.69 | 5.55 | 0.08 | 0.23 | 0.73 |

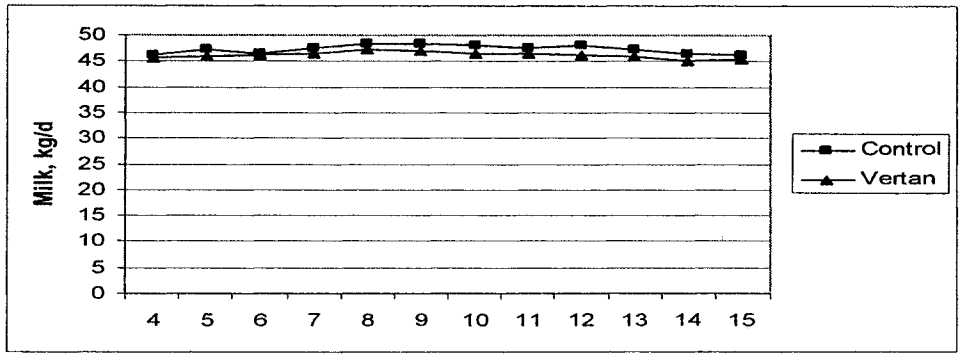
[1]The highest of the two SE were used.
[2]Fat corrected milk.
[3]Energy corrected milk.
[4]Solids not fat.

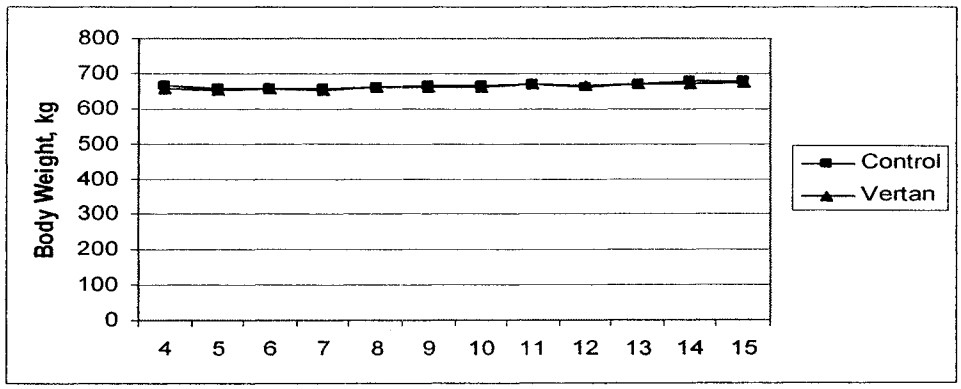
Table 5. Effect of feeding VERTAN to lactating Holstein cows on BW, BCS, BUN concentrations, rumen $NH_3$-N concentrations, and rumen pH.

| | Treatment | | | P-value | |
|---|---|---|---|---|---|
| Item | Control | VERTAN | SE[1] | Treatment | Trt*Week |
| BW, kg | 664 | 662 | 3.24 | 0.56 | 0.90 |
| BCS | 2.92 | 2.97 | 0.07 | 0.60 | 0.48 |
| BUN | 25.3 | 26.0 | 0.78 | 0.52 | 0.36 |
| Rumen parameters | | | | | Trt*Hour |
| pH | 5.99 | 6.0 | 0.02 | 0.86 | 0.00 |
| $NH_3$-N, mg/dl | 14.8 | 13.8 | 0.60 | 0.24 | <.0001 |

[1]The highest of the two SE were used.



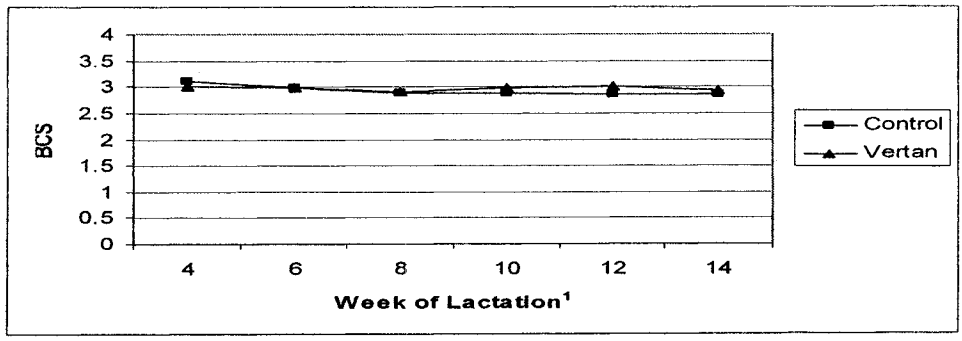
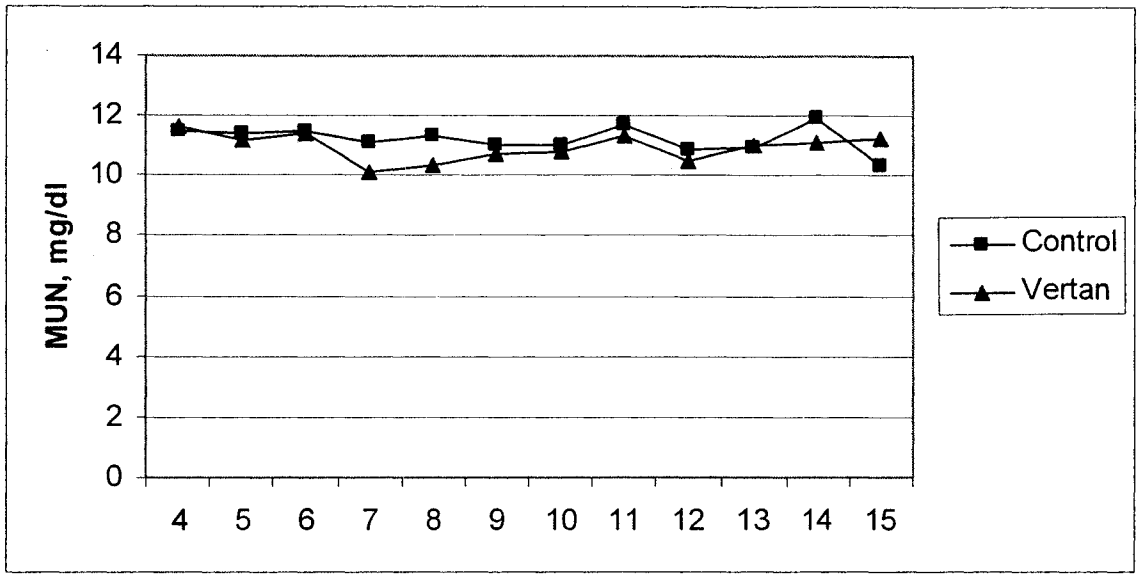




Figure 1. Effect of feeding VERTAN to lactating Holstein cows on milk yield, DMI, BW, and BCS.
[1]Measurements were not taken week 1 and 2. Week 3 was used as covariate.



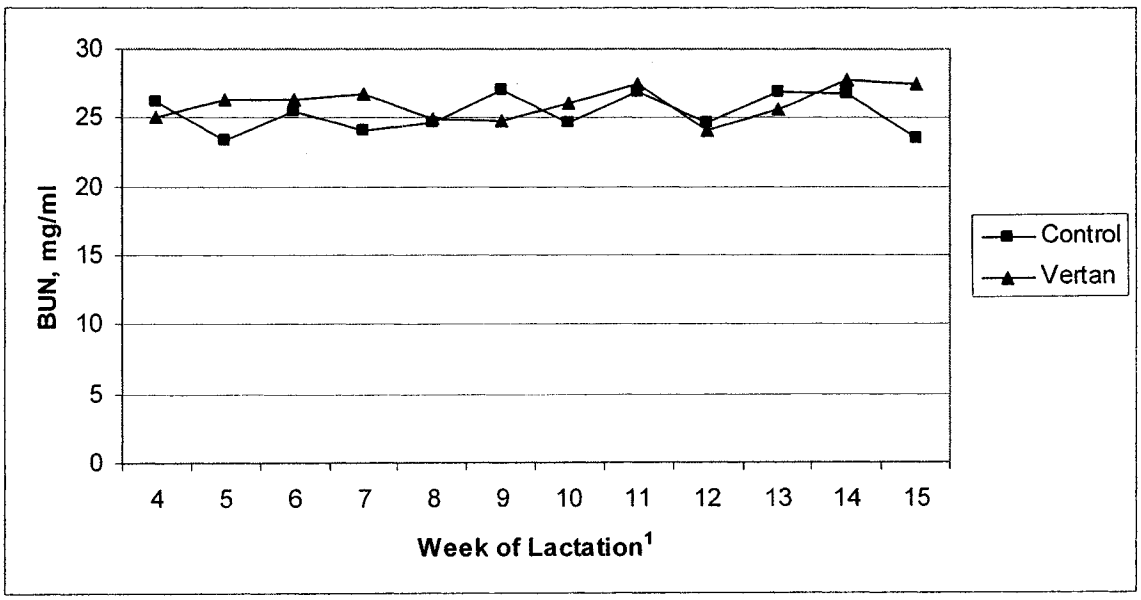




Figure 2. Effect of feeding VERTAN to lactating Holstein cows on BUN and MUN.
[1]Measurements were not taken week 1 and 2. Week 3 was used as covariate.

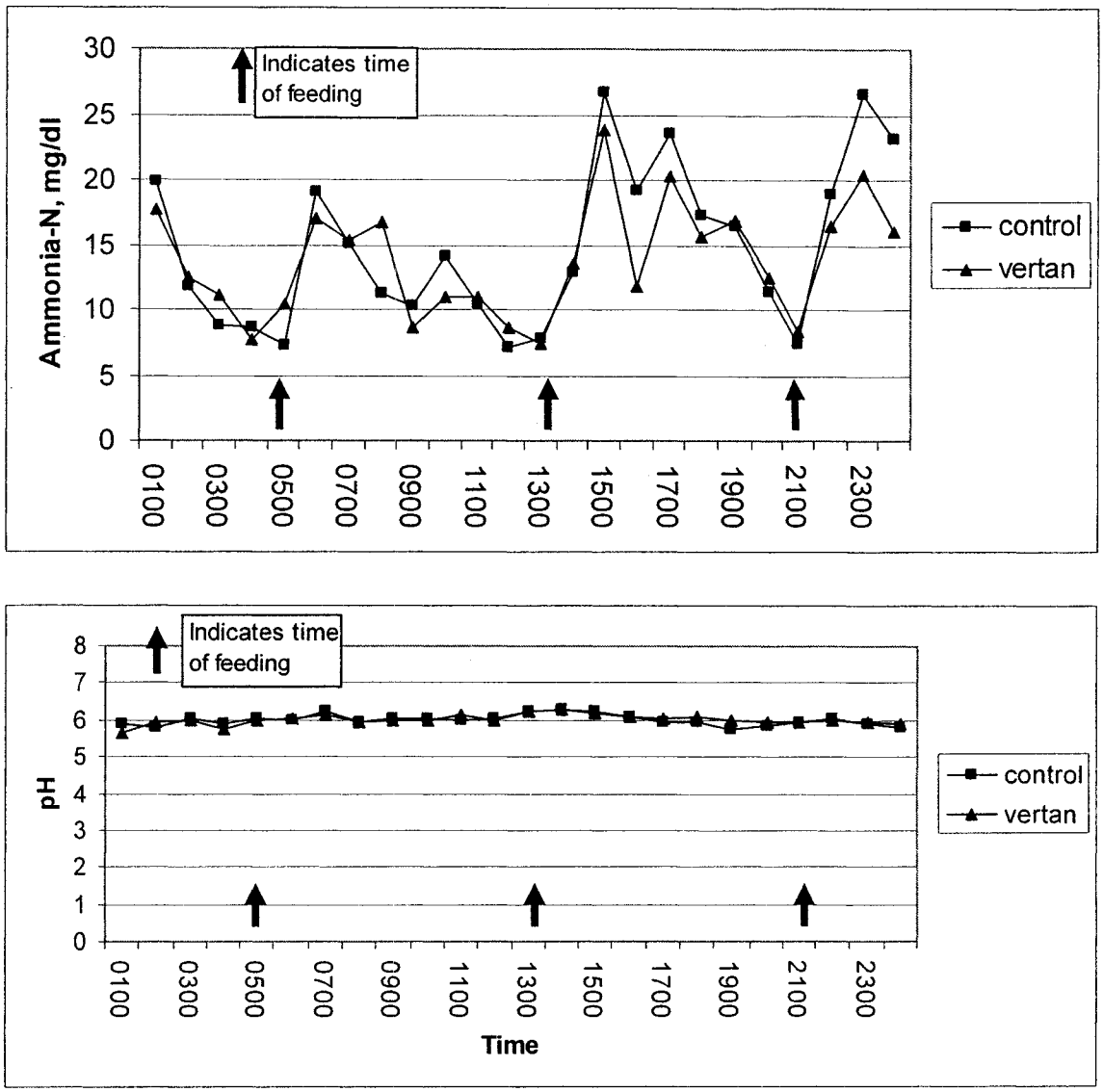



Figure 3. Effect of feeding VERTAN to lactating Holstein cows on rumen pH and $NH_3$-N concentrations over a 24-h period.

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# APPENDIX

UNIVERSITY of NEW HAMPSHIRE

June 14, 2005

Schwab, Charles
Animal & Nutritional Sciences
22A Colovos Rd, NACS Center
Durham, NH 03824

**IACUC #:** 050507
**Approval Date:** 05/20/2005
**Review Level:** D
**Project:** Effects of Essential Oils on Protein Metabolism

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

**Please Note:**

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger E. Wells, D.V.M.
Vice Chair

cc: File

**Research Conduct and Compliance Services, Office of Sponsored Research, Service Building, 51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564**